30. (Twice Amended) A method [of inducing immunoprotection] for inducing an immune response in a warm-blooded animal comprising

administering to the animal a [vaccine] composition comprising a [microbial] bacterial cell, the bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising [at least one of the following nucleic acid sequences]

[(a)] an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal[; and] ,and wherein the essential gene is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell.

[(b) a lethal gene, wherein expression of the essential gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.]

33. (Amended) The method of claim 30, wherein the [vaccine] composition is administered to mucosal surfaces of the animal.

35. (Amended) The method of claim [30] 39, wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, wherein the cell is a member of the *Enterobacteriaceae*.

36. (Amended) The method of claim 30, wherein the [microbial] bacterial cell is a member of the *Enterobacteriaceae*.

37. (Amended) The method of claim 36, wherein the [microbial] bacterial cell is an avirulent *Salmonella*.

39. (Amended) The method of claim 30, wherein the [cell comprises both an essential gene and a lethal gene] system further comprises a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

Claim 40 is cancelled.

C6

41. (Amended) The method of claim [40] 30, wherein the essential gene encodes an enzyme which catalyzes the biosynthesis of the cell wall and its precursors.

C7

44. (Amended) The method of claim [40] 30, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dal*, *ddl*, *fab*, *fad*, *pls*, a gene encoding a modification methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

C8

48. (Amended) The method of claim [30] 39, wherein expression of the essential gene or the lethal gene is regulated by a trans regulatory element.

50. (Amended) The method of claim [30] 39, wherein expression of the essential gene or the lethal gene is regulated by using promoters or regulatory elements that are regulated by temperature, or by other regulatory systems adapted to function in a temperature-dependent manner.

C9

51. (Amended) The method of claim 50, wherein the essential gene is regulated by being operatively linked to [regulation selected from the group consisting of a *virB* promoter with a *virF* gene and promoter elsewhere in the cell, a *virF* positive activator in combination with promoters of a *yopH* gene or a *yadR* gene] either

(a) a *virB* promotor, wherein the bacterial cell further comprises a *virF* gene and promoter; or

(b) a *virF* positive activator in combination with a promoter of a *yopH* gene or a *yadR* gene.

C10

66. (New) A method for inducing an immune response in a warm-blooded animal comprising administering to the animal a composition comprising a bacterial cell, the bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising an essential gene, wherein

(a) expression of the essential gene in the cell is essential to the viability of the cell;

(b) the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(c) the essential gene is a copy of a native gene.

67. (New) The method of claim 66, wherein the composition further comprises an expression gene wherein the expression gene encodes an antigen.

68. (New) The method of claim 67, wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens and non-insect animal antigens.

69. (New) The method of claim 66, wherein the composition is administered to mucosal surfaces of the animal.

70. (New) The method of claim 66; wherein the bacterial cell is a member of the *Enterobacteriaceae*.

71. (New) The method of claim 70, wherein the bacterial cell is an avirulent *Salmonella*.

72. (New) The method of claim 66, wherein the essential gene is an *asd* gene.

73. (New) The method of claim 66, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dal*, *ddl*, *fab*, *fad*, *pls*, a gene encoding a modification methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

74. (New) The method of claim 66, wherein the system further comprises a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

75. (New) The method of claim 74, wherein the lethal gene is selected from the group consisting of a member of the *gef* gene family, a plasmid maintenance gene, a gene encoding a nuclease, a gene encoding a phospholipase, a gene encoding an endolysin, a gene encoding a holin, and a gene encoding a tRNA with a wrong codon.

76. (New) The method of claim 74, wherein the lethal gene is the combination of bacteriophage P22 lysis genes 13 and 19.

CLAIMS AS AMENDED

30. (Twice Amended) A method for inducing an immune response in a warm-blooded animal comprising administering to the animal a composition comprising a bacterial cell, the bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and wherein the essential gene is essential for metabolism, growth, cell wall integrity or cell membrane integrity of the bacterial cell.

33. (Amended) The method of claim 30, wherein the composition is administered to mucosal surfaces of the animal.

35. (Amended) The method of claim 39, wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, wherein the cell is a member of the *Enterobacteriaceae*.

36. (Amended) The method of claim 30, wherein the bacterial cell is a member of the *Enterobacteriaceae*.

37. (Amended) The method of claim 36, wherein the bacterial cell is an avirulent *Salmonella*.

39. (Amended) The method of claim 30, wherein the system further comprises a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

41. (Amended) The method of claim 30, wherein the essential gene encodes an enzyme which catalyzes the biosynthesis of the cell wall and its precursors.

44. (Amended) The method of claim 30, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dal*, *ddl*, *fab*, *fad*, *pls*, a gene encoding a modification

methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

48. (Amended) The method of claim 39, wherein expression of the essential gene or the lethal gene is regulated by a trans regulatory element.

50. (Amended) The method of claim 39, wherein expression of the essential gene or the lethal gene is regulated by using promoters or regulatory elements that are regulated by temperature, or by other regulatory systems adapted to function in a temperature-dependent manner.

51. (Amended) The method of claim 50, wherein the essential gene is regulated by being operatively linked to either

(a) a *virB* promotor, wherein the bacterial cell further comprises a *virF* gene and promoter; or

(b) a *virF* positive activator in combination with a promoter of a *yopH* gene or a *yadR* gene.

66. (New) A method for inducing an immune response in a warm-blooded animal comprising administering to the animal a composition comprising a bacterial cell, the bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising an essential gene, wherein

(a) expression of the essential gene in the cell is essential to the viability of the cell;

(b) the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(c) the essential gene is a copy of a native gene.

67. (New) The method of claim 66, wherein the composition further comprises an expression gene wherein the expression gene encodes an antigen.

68. (New) The method of claim 67, wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens and non-insect animal antigens.

69. (New) The method of claim 66, wherein the composition is administered to mucosal surfaces of the animal.

70. (New) The method of claim 66, wherein the bacterial cell is a member of the *Enterobacteriaceae*.

71. (New) The method of claim 70, wherein the bacterial cell is an avirulent *Salmonella*.

72. (New) The method of claim 66, wherein the essential gene is an *asd* gene.

73. (New) The method of claim 66, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapC*, *dapD*, *dapE*, *dal*, *dil*, *fab*, *fad*, *pls*, a gene encoding a modification methylase, a gene encoding a DNA ligase, a gene encoding a DNA gyrase, and a gene encoding a phospholipase.

74. (New) The method of claim 66, wherein the system further comprises a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

75. (New) The method of claim 74, wherein the lethal gene is selected from the group consisting of a member of the *gef* gene family, a plasmid maintenance gene, a gene encoding a nuclease, a gene encoding a phospholipase, a gene encoding an endolysin, a gene encoding a holin, and a gene encoding a tRNA with a wrong codon.

76. (New) The method of claim 74, wherein the lethal gene is the combination of bacteriophage P22 lysis genes 13 and 19.